Amendments to the Specification:

Please replace the paragraph beginning at page 8, line 4, with the following rewritten paragraph:

Using substantially the process of US 6,180,357, the mouse monoclonal antibody H460-16-2 was obtained following immunization of mice with cells from a patient's lung tumor biopsy. The H460-16-2 antigen was expressed on the cell surface of a broad range of human cell lines from different tissue origins. The breast cancer cell line MDA-MB-231 (MB-231) was only 1 of 2 cancer cell lines tested that was susceptible to the cytotoxic effects of H460-16-2.

Please replace the Table beginning at page 38, line 1, with the following rewritten Table:

Sec. No.	Age	Sex	Organ	Diagnosis	H460-16-2	Vimentin	Negative Control
1	59	M	Skin	Malignant melanoma	+++ M	+++ M/C	-
2	25	F	Skin	Squamous cell carcinoma	-	+++ M/C	-
3	50	F	Breast	Infiltrating ductal carcinoma	+ Tumor, +++ Stroma	++ Stroma	-
4	57	F	Breast	Invasive papillary carcinoma	+/-	++ Stromal fibroblasts, Blood vessels	-
5	35	F	Breast	Infiltrating lobular carcinoma	+/-	CS	-
6	40	M	Lymph node	Malignant lymphoma, immunoplastic	+++ M	+++ M/C	-
7	58	M	Lymph node	Metastatic adenoca from stomach	+/-	+++ Tumor, Lipocytes	-
8	53	F	Bone	Osteosarcoma	+ M/C	+++ M/C	-
9	26	M	Bone	Giant cell tumor	+ M/C	++ M/C	-
10	40	M	Bone	Chondrosarcoma	CS	CS	CS
11	51	F	Soft tissue	Liposarcoma	-	+++ M/C	-
12	47	F	Soft tissue	Neurofibromatosis	+ M/C	+++ M/C	-
13	74	M	Nasal cavity	Inverted papilloma	++ M	+ Keratin	-
14	57	M	Larynx	Squamous cell carcinoma	+++ M	+++ Stroma	-
15	60	M	Lung	Adenocarcinoma	+/-	++ M/C	-
16	51	F	Lung	Squamous cell carcinoma	+++ M/C	+++ M/C	-
17	68	F	Lung	Adenocarcinoma	+/-	+++ M/C	-
18	60	M	Lung	Small cell carcinoma	+/-	+++ M/C	-
19	88	F	Tongue	Squamous cell carcinoma	+++ M	+++ Stroma	-
20	34	F	Parotid gland	Pleomorphic adenoma	-	++ M/C	-
21	50	F	Parotid gland	Warthin tumor	+++ M/C	+++ Tumor, Lymphocytes	-
22	40	F	Parotid gland	Pleomorphic adenoma	++ M/C	+++ M/C	-
23	56	M	Submandibular gland	Salivary duct carcinoma	-	+++ M/C	-
24	69	F	Liver	Cholangiocarcinoma	+/-	+/- Tumor, +++ Blood vessels	-
25	51	M	Liver	Metastatic gastric carcinoma	-	++ Stroma	-
26	64	M	Liver	Hepatocellular carcinoma	+/-	+/-	-
27	62	F	Gall bladder	Adenocarcinoma	++ Tumor, Lympocytes	+ Stroma	-
28	64	F	Pancreas	Adenocarcinoma	++ M/C	++ Stroma	-
29	68	M	Esophagus	Squamous cell carcinoma	+/-	++ Stroma	-
30	73	M	Stomach	Adenocarcinoma, poorly differentiated	+ M/C	++ Stroma, Blood vessels	-
31	63	M	Stomach	Adenocarcinoma, moderately differentiated	++ M/C	++ M/C	-
32	59	F	Stomach	Signet ring cell carcinoma	++ M/C	++ M/C	-
33	62	M	Stomach	Malignant lymphoma	+++ M/C	+++ M/C	-
34	51	M	Stomach	Borderline stromal tumor	-	++ M/C	-
35	42	M	Small intestine	Malignant stromal tumor	-	+++ M/C	-
36	52	F	Appendix	Pseuomyxoma peritonii	-	+ Tumor, +++ Lipocytes	-
37	53	M	Colon	Adenocarcinoma	+ M/C	++ Stroma	-
38	67	M	Rectum	Adenocarcinoma	++ M	++ Lipocytes, Blood vessels	-
39	75	F	Kidney	Transitional cell carcinoma	+ M/C	++ Stroma	-
40	54	F	Kidney	Renal cell carcinoma	+/-	++ M	-
41	75	F	Kidney	Renal cell carcinoma	+/-	+ Tumor, +++ Stroma	-
42	65	M	Urinary bladder	Carcinoma, poorly differentiated	++ M/C	++ Stroma	-
43	67	M	Urinary bladder	Transitional cell carcinoma, high grade	-	+++ Stroma, Blood vessels	-
44	62	M	Prostate	Adenocarcinoma	+++ M	+++ Tumor, Stroma, Blood vessels	-
45	30	M	Testis	Seminoma	+/-	+++ Blood vessels	-
46	68	F	Uterus	Endometrial adenocarcinoma	++ Stroma	+ Tumor, +++ Stroma	-
47	57	F	Uterus	Leimyosacoma	+ PS	+ M/C	-
48	45	F	Uterus	Leiomyoma	+ C	+++ M/C	-
49	63	F	Uterine cervix	Squamous cell carcinoma	+++ M	+/- Tumor, ++ Stroma	-
50	12	F	Ovary	Endodermal sinus tumor	-	++ Tumor, Stroma	-
51	33	F	Ovary	Mucinous adenocarcinoma	-	++ Stroma	-
52	70	F	Ovary	Fibrothecoma	-	+++ M/C	-
53	67	F	Adrenal gland	Cortical carcinoma	-	+++ M/C	-
54	61	F	Adrenal gland	Pheohromcytoma	-	+++ M/C	-
55	54	M	Thyroid	Papillary carcinoma	++ M/C	+/- Tumor, ++ Stroma	-
56	58	F	Thyroid	Follicular carcinoma, minimally invasive	++ M	+++ M/C	-
57	74	M	Thymus	Thymoma	+/-	++ M/C	-
58	66	F	Brain	Meningioma	-	+++ M/C	-
59	62	M	Brain	Glioblastoma multiforme	+++ M	++ Tumor, Blood vessels	-

Please replace the heading "Summary of the Invention:" beginning at page 6, line 14, with the following heading:

Brief Summary of the Invention:

Please delete the following verbiage beginning at page 8, line 4 thru page 15, line 8, and reinsert it beginning at page 18, line 3, after the heading "Detailed Description Of The Invention:" :

Using substantially the process of US 6,180,357, the mouse monoclonal antibody H460-16-2 was obtained following immunization of mice with cells from a patient's lung tumor biopsy. The H460-16-2 antigen was expressed on the cell surface of a broad range of human cell lines from different tissue origins. The breast cancer cell line MDA-MB-231 (MB-231) was only 1 of 2 cancer cell lines tested that was susceptible to the cytotoxic effects of H460-16-2.

The result of H460-16-2 cytotoxicity against MB-231 cells in culture was further extended by its anti-tumor activity towards these cells when transplanted into mice. In an *in vivo* model of breast cancer, the human MB-231 cells were implanted underneath the skin at the scruff of the neck of immunodeficient mice, as they are incapable of rejecting the human tumor cells due to a lack of certain immune cells. Pre-clinical xenograft tumor models are considered valid predictors of therapeutic efficacy. Xenografts in mice grow as solid tumors developing stroma, central necrosis and neo-vasculature. The mammary tumor cell line MB-231 has been evaluated as an *in vivo* xenograft model in immuno-deficient mice. The good engraftment or 'take-rate' of the MB-231 tumors and the sensitivity of the tumors to standard chemotherapeutic agents have characterized it as a suitable model. The parental cell line and variants of the cell line have been used in xenograft tumor models to evaluate a wide range of therapeutic agents.

In the preventative *in vivo* model of human breast cancer, H460-16-2 was given to mice one day prior to implantation of tumor cells followed by weekly injections for a period of 7 weeks. H460-16-2 treatment was significantly ($p<0.0001$) more effective in suppressing tumor growth

during the treatment period than an isotype control antibody, which was identical to H460-16-2 in structure and size but incapable of binding MB-231 cells. At the end of the treatment phase, mice given H460-16-2 had tumors that grew to only 1.3 percent of the control group. During the post treatment follow-up period, the treatment effects of H460-16-2 were sustained and the mean tumor volume in the treated groups continued to be significantly smaller than controls until the end of the measurement phase. Using survival as a measure of antibody efficacy, it was estimated that the risk of dying in the H460-16-2 treatment group was about 71 percent of the antibody buffer control group ($p=0.028$) at 70 days post-treatment. These data demonstrated that H40-16-2 treatment conferred a survival benefit compared to the control-treated groups. H460-16-2 treatment appeared safe, as it did not induce any signs of toxicity, including reduced body weight and clinical distress. Thus, H460-16-2 treatment was efficacious as it both delayed tumor growth and enhanced survival compared to the control-treated groups in a well-established model of human breast cancer. These results were also reproducible as similar findings were observed in another study of this kind and suggest its relevance and benefit to treatment of people with cancer.

Besides the preventative *in vivo* tumor model of breast cancer, H460-16-2 demonstrated anti-tumor activity against MB-231 cells in an established *in vivo* tumor model. In this xenograft tumor model, MB-231 breast cancer cells were transplanted subcutaneously into immunodeficient mice such that the tumor reached a critical size before antibody treatment. Treatment with H460-16-2 was compared to the standard chemotherapeutic drug, cisplatin, and it was shown that the cisplatin and H460-16-2 treatment groups had significantly ($p<0.001$) smaller mean tumor volumes compared with groups treated with either antibody dilution buffer or the isotype control antibody. H460-16-2 treatment mediated tumor suppression that was approximately two-thirds that of

cisplatin chemotherapy but without the significant weight loss ($p<0.003$) and clinical distress observed with cisplatin. The anti-tumor activity of H460-16-2 and its minimal toxicity make it an attractive anti-cancer therapeutic agent.

In the post-treatment period, H460-16-2 showed a significant survival benefit ($p<0.02$) as the risk of dying in the H460-16-2 group was about half of that in the isotype control antibody group at >70 days after treatment. The observed survival benefit continued on at 120 days post-treatment where 100 percent of the isotype control and cisplatin treated mice had died compared to 67 percent of the H460-16-2 treatment group. H460-16-2 maintained tumor suppression by delaying tumor growth by 26 percent compared to the isotype control antibody group. At 31 days post treatment, H460-16-2 limited tumor size by reducing tumor growth by 48 percent compared to the isotype control group, which is comparable to the 49 percent reduction observed at the end of the treatment. In the established tumor model of breast cancer, these results indicate the potential of H460-16-2 to maintain tumor suppression beyond the treatment phase and demonstrates the ability of the antibody to reduce the tumor burden and enhance survival in a mammal.

By immunohistochemistry (IHC) staining, sections of mouse tissues from multiple organs were stained with H460-16-2 to localize the H460-16-2 antigen within individual cell types of various tissues. Consistent with the tumor suppressive effects of H460-16-2 against MB-231 cells *in vivo*, the H460-16-2 antigen was strongly expressed on sections of tumor tissue harvested from untreated mice subcutaneously implanted with MB-231 cells. Expression of the H460-16-2 antigen in normal mouse tissues is required for supporting the mouse as an appropriate model of toxicity for H460-16-2. It was observed that the H460-16-2 antigen had a limited expression pattern in the

mouse as it was only expressed in the kidney and ovary. In order to validate the mouse as a suitable model for toxicity, there needs to be similar antigen expression in normal human tissue.

For clinical trials and to validate an appropriate animal model for toxicity, the specificity of H460-16-2 towards normal human tissues was determined. By IHC staining with H460-16-2, the majority of the tissues failed to express the H460-16-2 antigen, including the vital organs, such as the liver, kidney, heart, and lung. H460-16-2 stained the skin, ureter, stomach and prostate, and strongly stained the salivary gland. Results from tissue staining indicated that H460-16-2 showed restricted binding to various cell types but had binding to infiltrating macrophages, lymphocytes, and fibroblasts. Therefore, the data indicate that the mouse is probably not the best model for toxicity since that although both the mouse and human show limited H460-16-2 tissue expression; the tissues positive for staining are not the same between the two species.

Localization of the H460-16-2 antigen and its prevalence within breast cancer patients is important in assessing the benefits of H460-16-2 immunotherapy to patients and designing effective clinical trials. To address H460-16-2 antigen expression in breast tumors from cancer patients, tumor tissue samples from 50 individual breast cancer patients were screened for expression of the H460-16-2 antigen. The results of the study showed that 64 percent of tissue samples stained positive for the H460-16-2 antigen. Expression of H460-16-2 within patient samples appeared specific for cancer cells as staining was restricted towards malignant cells. In contrast, H460-16-2 stained 2 of 9 samples of normal tissue from breast cancer patients. Breast tumor expression of the H460-16-2 antigen appeared to be mainly localized to the cell membrane of malignant cells, making it an attractive target for therapy. H460-16-2 expression was further evaluated based on breast tumor expression of the receptors for the hormones estrogen and progesterone, which play an

important role in the development, treatment, and prognosis of breast tumors. No correlation was apparent between expression of the H460-16-2 antigen and expression of the receptors for either estrogen or progesterone. When tumors were analyzed based on their stage, or degree to which the cancer advanced, results suggested a trend towards greater positive expression with higher tumor stage, but the results were limited by the small sample size.

To further extend the potential therapeutic benefit of H460-16-2, the frequency and localization of the antigen within various human cancer tissues was determined. Several cancer types, besides breast, were positive for the H460-16-2 antigen. The positive human cancer types included skin (½), lung (4/4), liver (2/3), stomach (4/5) and kidney (3/3). Some cancers did not express the antigen; these included ovary (0/3), adrenal gland (0/2) and small intestine (0/1). As with human breast tumor tissue, localization occurred predominately on the membrane of tumor cells. So, in addition to the H460-16-2 antibody binding to cancer cell lines *in vitro*, there is evidence that the antigen is expressed in humans, and on multiple types of cancers. In toto, this data demonstrates that the H460-16-2 antigen is a cancer associated antigen and is expressed in humans, and is a pathologically relevant cancer target. Further, this data also demonstrates the binding of the H460-16-2 antibody to human cancer tissues, and can be used appropriately for assays that can be diagnostic, predictive of therapy, or prognostic. In addition, the cell membrane localization of this antigen is indicative of the cancer status of the cell due to the lack of expression of the antigen in most non-malignant cells, and this observation permits the use of this antigen, its gene or derivatives, its protein or its variants to be used for assays that can be diagnostic, predictive of therapy, or prognostic.

Preliminary data indicate that the antigen recognized by H460-16-2 could be a variant of the tumor rejection antigen known as the 96 kDa heat shock protein (gp96). This is supported by biochemical studies showing that monoclonal antibodies reactive against gp96 identify proteins that were bound to H460-16-2. By IHC analysis of mouse tissues using H460-16-2 and anti-gp96 antibodies, the gp96 antigen appeared to be more widely expressed than the H460-16-2 antigen. These results were similar to those for IHC staining of normal human tissues as the H460-16-2 antigen was expressed on a smaller subset of cells compared to the gp96 antigen. IHC analysis of human breast tumor tissues indicated that the gp96 antigen was more prevalent with approximately 84 percent of samples staining positive with the anti-gp96 antibody. The gp96 antigen was also expressed differently than H460-16-2 as it showed both high cytoplasmic and cell membrane localization. These results thus suggest that H460-16-2 may be a variant of gp96.

In all, this invention teaches the use of the H460-16-2 antigen as a target for a therapeutic agent, that when administered can reduce the tumor burden of a cancer expressing the antigen in a mammal, and can also lead to a prolonged survival of the treated mammal. This invention also teaches the use of a CDMAB (H460-16-2), and its derivatives, to target its antigen to reduce the tumor burden of a cancer expressing the antigen in a mammal, and to prolong the survival of a mammal bearing tumors that express this antigen. Furthermore, this invention also teaches the use of detecting the H460-16-2 antigen in cancerous cells that can be useful for the diagnosis, prediction of therapy, and prognosis of mammals bearing tumors that express this antigen.

If a patient is refractory to the initial course of therapy or metastases develop, the process of generating specific antibodies to the tumor can be repeated for re-treatment. Furthermore, the anti-cancer antibodies can be conjugated to red blood cells obtained from that patient and re-infused

for treatment of metastases. There have been few effective treatments for metastatic cancer and metastases usually portend a poor outcome resulting in death. However, metastatic cancers are usually well vascularized and the delivery of anti-cancer antibodies by red blood cells can have the effect of concentrating the antibodies at the site of the tumor. Even prior to metastases, most cancer cells are dependent on the host's blood supply for their survival and anti-cancer antibody conjugated to red blood cells can be effective against *in situ* tumors as well. Alternatively, the antibodies may be conjugated to other hematogenous cells, e.g. lymphocytes, macrophages, monocytes, natural killer cells, etc.

There are five classes of antibodies and each is associated with a function that is conferred by its heavy chain. It is generally thought that cancer cell killing by naked antibodies are mediated either through antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). For example murine IgM and IgG2a antibodies can activate human complement by binding the C-1 component of the complement system thereby activating the classical pathway of complement activation which can lead to tumor lysis. For human antibodies, the most effective complement activating antibodies are generally IgM and IgG1. Murine antibodies of the IgG2a and IgG3 isotype are effective at recruiting cytotoxic cells that have Fc receptors which will lead to cell killing by monocytes, macrophages, granulocytes and certain lymphocytes. Human antibodies of both the IgG1 and IgG3 isotype mediate ADCC.

Another possible mechanism of antibody mediated cancer killing may be through the use of antibodies that function to catalyze the hydrolysis of various chemical bonds in the cell membrane and its associated glycoproteins or glycolipids, so-called catalytic antibodies.

There are two additional mechanisms of antibody mediated cancer cell killing which are more widely accepted. The first is the use of antibodies as a vaccine to induce the body to produce an immune response against the putative antigen that resides on the cancer cell. The second is the use of antibodies to target growth receptors and interfere with their function or to down regulate that receptor so that effectively its function is lost.